in a median of 144 days. Grade II acute GvHD occurred in 3 of 8 engrafting patients. No patient developed extensive chronic GvHD. Five patients did not survive; causes of death were graft failure with viral infection in 3 children and viral infection in 2. Seven children 58% are surviving for 6–43 months (median, 24 months) posttransplantation. All surviving children have shown stabilization of disease progression. Children transplanted before age 3 years have continued to gain developmental skills including speech, albeit at a slower-than-normal pace. All children exhibit some degree of autistic behavior but remain relatively calm and happy without major sleep disturbances or aggressive behaviors. Four of the surviving children are now over 4.5 years old, and all appear to have higher neurocognitive functioning than untreated Sanfilippo patients of similar age. We conclude that UCBT improves the quality of life for patients with Sanfilippo syndrome. Transplantation before age 2 years allows for continued cognitive and developmental gains.

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REDUCED TOXICITY MYELOABLATIVE CONDITIONING REGIMEN FOR CHILDREN WITH MARROW STEM CELL DEFECTS (MSCD) OR LEUKEMIA

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Two major problems with busulfan/cyclophosphamide-containing conditioning regimens for children are acute toxicities (mucositis and VOD) and graft failure/rejection. We have prospectively evaluated a conditioning regimen using targeted dosing of IV busulfan, fludarabine, and rabbit ATG for its toxicity and efficacy. Patients included 19 children receiving HLA-matched related or unrelated transplantation for MSCD, including thalassemia, aplastic anemia (AA), metabolic diseases, congenital neutropenia, and primary immunodeficiencies (n = 15), and leukemia/MDS (n = 3). The targeted average steady-state concentration (Css) of busulfan was 600 ng/mL for the cases of MSCD and 900 ng/mL for the cases of leukemia/MDS. Donors were well HLA-matched unrelated volunteers (n = 11), unrelated cord blood (n = 1), and HLA-matched relatives (n = 6). The most common toxicity was mucositis, which developed in 1/3 of the patients. None of the patients developed VOD. All patients showed marrow engraftment by 28 days. However, 2 of 19 patients, 1 with AA and the other with adrenoleukodystrophy, rejected the graft by 6 weeks posttransplantation. Two additional patients, 1 with mannosidosis and 1 with thalassemia, had graft failure 6 months posttransplantation. Three of these 4 were recipients of matched unrelated donor marrow grafts, and 1 received related bone marrow graft. Three of the 4 patients were successfully retransplanted using a TBI-containing regimen. There were 2 deaths, 1 from a preexisting CMV infection in a child with Wiskott-Aldrich syndrome and 1 from complications related to graft rejection. The long-term disease-free survival was 89% ± 7%, with a median follow-up of 27 months (range, 4–41 months), including the patients that needed second transplants. The durable engraftment rate was ~80%. We established a quantitative PCR assay using short-tandem repeats (STRs) to monitor engraftment in blood and bone marrow in cell subsets including CD3, CD19, CD14/15, and CD34. Full (n = 6) or partial (n = 9) donor chimerism was achieved in 15 evaluable patients. Neither the results of early engraftment studies (< 30 days) nor the percentasge of donor CD3+ cells were predictive of ultimate engraftment outcome. Five of 9 patients with durable mixed chimerism had < 50% donor CD3+ cells from 123–420 days posttransplantation. These results indicate that substituting fludarabine for cyclophosphamide can reduce toxicity with respect to both mucositis and VOD, but that alternative regimens need to be developed to improve durable engraftment.

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LOW TRANSPLANT-RELATED MORTALITY AFTER STEM CELL TRANSPLANTATION WITH IV BUSULFAN-BASED CONDITIONING IN PEDIATRIC PATIENTS

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High-dose busulfan is widely used in allogeneic and autologous marrow transplantation preparative regimens. We evaluated the safety and toxicity of IV busulfan (Bu) in combination with variety of chemotherapeutic agents, including cytoxan, cytoxan +VP16, melphalan, and TBI+Bu+VP−16, as preparative therapy for stem cell transplantation in pediatric patients. The length of follow up study was 3–40.3 months. Twenty-five pediatric patients (a total of 30 transplantation procedures) with hematologic malignancies (n = 11), solid tumors (n = 6), and nonmalignant disorders (n = 8) were evaluated. Six patients underwent autologous transplantation (5 with tandem transplantation), and 19 patients underwent HLA-matched sibling allogeneic transplantation. The sources of the stem cells were peripheral stem cells in 21, bone marrow in 7, and cord blood in 2.

All but 2 patients engrafted. ANC 500 was achieved at day 10–25 (median, day 13), and platelets > 20 $\times 10^9$ were achieved at day 14–85 (median, day 24). Grade 3–4 liver toxicities were diagnosed in 17.3% of all patients; grade 3–4 pulmonary toxicities, in 10.9%. There was no mortality in the first 100 days after transplantation. Pharmakokinetic studies showed a wide variability of busulfan levels. The mean AUCs achieved after administration of the initial test dose was 848.0 (461.1–1300.4). Adjustment of the IV busulfan dose was made in 7 patients (28%). All doses were increased.

Individualization of busulfan doses has been recommended to obtain sufficient dose intensity to achieve engraftment while avoiding relapse and toxicity. Published data demonstrated that using the IV formulation of busulfan reduces variability in busulfan levels in both adult and pediatric patients. However, most of the studies evaluated IV busulfan in combination with cytoxan. We found that IV busulfan in pediatric patients is generally well tolerated in combination with cytoxan, melphalan, TBI, VP-16, and fludarabine.

SOLID TUMORS

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TARGETED RADIOTHERAPY TO THE SKELETON USING 166HO-DOTMP WITH AUTOLOGOUS STEM CELL TRANSPLANTATION FOR PATIENTS WITH BONE-ONLY METASTATIC BREAST CANCER

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The skeleton is the most frequent site of metastatic disease in breast cancer. A subset of patients develop bone-dominant disease without metastases to other organs. An attractive strategy is to utilize bone-targeted radiotherapy. This study was designed to determine the safety and preliminary efficacy of 166Ho-DOTMP, which localizes to bone, providing radiation to adjacent marrow and malignant cells. The limiting toxicity is myelosuppression, which can be overcome by autologous stem cell transplantation. We studied this strategy in patients with breast cancer metastatic to confined to the bone.

Treatment occurred between 1997 and 1999. Patients were eligible if they were < 65 years of age, had bone-only disease, had an estrogen receptor–negative tumor, or had failed hormonal therapy. A 30-mCi tracer dose was administered to assess targeting and to calculate the therapeutic dose. The therapeutic dose was administered in one of 2 dose levels, either 22 Gy (n = 3) or 28 Gy (n = 3). Treatment was followed by autologous stem cell transplantation when the ongoing radiation dose to marrow was < 1 cGy/hour. The 6 subjects received a median of 1548 mCi (range, 870–2065 mCi) of 166Ho DOTMP. There was selective uptake in the bone, particularly in osteoblastic metastases; unbound drug was excreted in the urine. The most common toxicities were mild nausea and vomiting. None of the patients experienced grade > 3 acute toxicity except for the expected profound myelosuppression. All patients had prompt hematologic recovery. Two patients developed hemorrhagic cystitis at approximately 2 years following therapy, which resolved in both cases. One of these patients also had GI bleeding and pseudomembranous colitis unrelated to the 166Ho DOTMP treatment. One patient developed MDS, but was found to have a preexisting trisomy-8 by FISH analysis from the periph-

eral blood stem cell product collected before treatment on this study. Two patients achieved complete remission and remain progression-free more than 5 years after study entry. Four relapsed and died of progressive disease; all recurred in extraosseous sites with 2 also having disease in the bone marrow. Median time to progression was 10 months.

We conclude that treatment with 166Ho DOTMP delivers targeted radiotherapy to the skeleton with an acceptable toxicity profile. Two patients with bone-only metastases achieved sustained complete responses. Further studies are warranted to evaluate this strategy for treatment of bone metastases.

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TANDEM HIGH-DOSE CHEMOTHERAPY (HDC) WITH PERIPHERAL BLOOD STEM CELL RESCUE (PBSCR) AS CONSOLIDATION THERAPY FOR HIGH-RISK (PRIMARY UNRESECTABLE PELVIC TUMORS OR METASTATIC DISEASE) EWING'S SARCOMA PATIENTS

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Methods: Between 1985 and 2004, 14 patients with high-risk Ewing's sarcoma received induction chemotherapy, surgery, and radiation therapy, with 8 of the 14 patients receiving tandem transplantations. The median age at diagnosis was 14 years (range, 1–17 years) for the 8 patients receiving PBSCR and 9 years (range, 4–14 years) for the 6 patients who did not receive PBSCR. Of the 6 patients who did not receive a stem cell rescue, 5 had primary pelvic tumors without metastatic disease, and 1 had a nonpelvic primary tumor with bone metastases. Of the 8 patients who received HDC and PBSCR, 6 patients had primary pelvic tumors (3 of them with pulmonary metastases), and the remaining 2 had primary bone disease outside of the pelvis with bone metastases. Of the 8 patients who received a transplant, 2 received chemotherapy only before PBSCR, and 6 received chemotherapy and surgery before transplantation. Three ablative regimens were used for the 8 patients. Six patients received tandem rescues. Rescue 1 included etoposide (VP-16), 800 mg/m^2/day for 3 days; carboplatin (CP), 667 mg/m^2/day for 3 days; and cyclophoshamide (CTX), 1800 mg/m^2/day for 2 days. Rescue 2 involved thiotepa (TT), 300 mg/m^2/day for 3 days; CTX (n = 1), melphalan/CTX (n = 4), and melphalan/TBI (n = 1); VP-16, 800 mg/m^2/day for 4 days; CTX, 1800 mg/m^2/day for 3 days; total body irradiation (TBI), 200 cGy BID for 3 days (n = 1); melphalan, 60 mg/m^2/day for 3 days; CTX, 500 mg/m^2/day for 4 days (n = 1). The median stem cell dose was 1.78 $\times$ 108 mnc/kg (range, 1.0 $\times$ 10^8 to 2.3 $\times$ 10^8 mnc/kg). **Results:** The mean time to achieve ANC $>$ 500 was 13 days (range, 7–17 days), and the mean time to achieve a platelet count $>$ 20K for 7 consecutive days unsupported was 23 days (range, 7–57 days). There were no toxic deaths in the 8 patients who received PBSCR. Five of the 8 patients who received PBSCR are in complete remission, with a median follow-up of 7.8 years (range, 36–118 months). Of the 6 patients who did not receive PBSCR, 3 continue in complete remission with a median follow-up of 10.5 years (range, 120–132 months). **Conclusions:** Our results suggest that high-dose therapy with PBSCR is an effective and feasible treatment for patients with high-risk Ewing's sarcoma. A larger longitudinal study would be helpful to further evaluate this treatment option.

STEM CELL BIOLOGY

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CD133-POSITIVE HEMATOPOIETIC STEM CELLS "STEMNESS" GENES CONTAIN MANY GENES MUTATED OR ABNORMALLY EXPRESSED IN LEUKEMIA

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Several groups have recently used microarray technology to study the common characteristics of stem cells from different tissues ("stemness") and the typical features of stem cells from various sources ("tissue specificity"). Most groups focused their study on mice, used relatively small cDNA microarrays, and used CD34 as the cell surface marker for hematopoietic stem cells isolation. We studied HSC cells from cord blood (CB) and peripheral blood (PB)characterized by expression of the primitive CD133 antigen, and used the Affymetrix Human Hu133A oligonucleotide arrays to study the gene expression profile of these cells.

An unsupervised hierarchical clustering of 14,025 "valid" probe sets showed a clear distinction between the CD133+ cells representing the stem cell population and CD133− cells that represent various stages of cell differentiation. Comparison of CD133+ cells isolated from CB and PB to CD133− cells identified 304 genes that were up-regulated by at least 2-fold in CB and 218 genes in PB. These genes were considered source-specific and possibly relevant to the unique properties of CB- and PB-derived HSC. A total of 244 genes were found to be up-regulated by at least 2-fold in the CD133+ cells of both CB and PB compared with the CD133− cells. Comparison of these "stemness" genes to the lists of "stemness" genes that were identified by 2 recent studies that analyzed mainly murine HSC identified totals of 33 and 65 common genes. Twenty-four genes were common to another study that analyzed human HSC.

Among these common "stemness" genes, we identified several groups of genes that have an important role in hematopoiesis: growth factor receptors, a group of transcription factors that includes several homeobox genes and TGF-β target genes, genes that have an important role in development, and genes involved in cell growth. Among these 4 groups, we identified 16 "stemness" genes (MPL, FLT3, HOXA9, MEIS 1, MLLT3, KIT, TIE, GATA-2, HOXA5, HOXA10, HLF, MYCN, EVI1, MYB, FHL1, and HMGA2) that are known to be mutated or abnormally regulated in acute leukemias. It can be suggested that key hematopoietic stemness machinery genes may lead to abnormal proliferation and leukemia on mutation or change of expression.

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PLEIOTROPHIN IS A KEY SWITCH OF HUMAN MONOCYTES AND BONE MARROW STEM CELLS INTO ENDOTHERLIAL-LIKE CELLS FOR ANGIOGENESIS IN TUMOR GROWTH

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Multiple myeloma (MM) patients express pleiotrophin (PTN), a secreted protein that binds CD138, and it is found at high levels in the serum of MM patients. We have discovered a novel mechanism leading to blood vessel formation by tumor cells. First, we purified human monocytes (CD14+) and cultured on collagen I, collagen IV, fibronectin, or laminin-coated dishes. The cells incubated on collagen I (but not on the other 3 proteins) with mCSF + PTN formed tube-like structures with positive staining for Flk-1. Complex lines consisting of multiple rows of elongated Flk-1+ cells in contact with each other were found. In contrast, monocytes incubated with only mCSF or on other substrates remained in separated positions. We also cloned human monocytic THP-1 cells with PTN sense or antisense whole sequencing DNA. We examined expression by RT-PCR of endothelial cell markers Flk-1, Tie-2, and vWf and monocyte markers *c-fms* and CD68. THP-1 cells infected with PTN sense strand expressed high amounts of Flk-1, Tie-2, and vWf similar to that found in human coronary artery endothelial cells and lost expression of *c-fms* and CD68. Next, we cultured THP1 monocytes with human myeloma RPMI8226 cells in transwell cultures, serum derived from MM patients with high serum levels of PTN, cell lines lacking PTN expression, or normal controls lacking serum PTN. The THP-1 cells exposed to the MM cell line or MM serum showed expression of endothelial markers. The expression of endothelial markers was blocked by adding anti-PTN antibody. We also determined whether PTN could also stimulate differentiation of bone marrow